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ABSTRACT

The chromosome complements of five species of hylid frogs are described and illustrated. One of the species, *Smilisca baudini*, is a "generalized" hylid and is used for comparative purposes. The other four species, *Pternohyla fodiens*, *Triprion petasatus*, *Hyla septentrionalis*, and *Hyla brunnea*, are among those having cephalic adaptations that are referred to as the casque-headed hylids. Three of these (*P. fodiens, T. petasatus*, and *H. septentrionalis*) have karyotypes that are indistinguishable from that of the generalized *S. baudini*: This includes a diploid number of 24 bi-armed chromosomes (12 pairs), of which six pairs are relatively large and six relatively small. The karyotype of the other casque-headed species is vastly different, however: *Hyla brunnea* has a diploid number of 34 chromosomes, of which 20 are uni-armed. Following a review of the chromosome variation known to occur within the Hylidae, it is inferred that the karyotype of *H. brunnea* was derived from an ancestral karyotype similar to that of *H. septentrionalis* by means of at least five whole-arm chromosomal aberrations.

INTRODUCTION

Among the approximately 450 species and 30 genera of frogs in the family Hylidae there are several groups of primarily Neotropical species with rather bizarre adaptations involving co-ossification of their skulls and cephalic dermis that qualify them for the appellation "casque-headed treefrogs." Trueb (1970) recently reviewed many of these frogs. She investigated their skull osteology in detail, compared them with species of hylids that lack cephalic co-ossification, and proposed a phylogenetic tree to illustrate their evolutionary relationships. Trueb selected *Smilisca baudini* as representative of a relatively unspecialized hylid for her comparisons.

The present report compares the chromosome morphology of four species of casque-headed hylid frogs for which karyotypes have been determined for the first time. In addition, the chromosomes are compared with the karyotype of the same generalized hylid selected by Trueb, *Smilisca baudini*, in an attempt to understand the significance of the chromosome variation observed.

Prior to this report, the only information in the literature pertaining to the chromosomes of the casque-headed species considered here was that *Pternohyla fodiens, Triprion petasatus*, and *Hyla septentrionalis* have a haploid number of 12 chromosomes (Duellman and Cole, 1965); the results presented here are consistent with those counts. A karyotype of *Smilisca baudini* similar to the one presented here was published recently by Bogart (1973).

METHODS

Chromosomes of 135 cells from bone marrow tissues of 20 frogs were examined on slides prepared for study by means of the colchicine, hypotonic citrate, flame dried procedures (Patton, 1967) as modified for amphibians (Cole and Leavens, 1971 [1972]). Chromosome terminology follows my earlier usage (Cole, 1970).

RESULTS

Smilisca baudini (Duméril and Bibron) has a diploid number of 24 chromosomes ($2n = 24$), of which 12 (pairs 1 through 6) are rather large and 12 (pairs 7 through 12) are relatively small (fig. 1A). This was determined by examining complete chromosome complements of 18 cells from two individuals (both males). Considering the six larger pairs of chromosomes in order of decreasing length, number 1 is metacentric to submetacentric, number 2 is submetacentric, numbers 3 and 4 are approximately equal in size but one pair (designated here as no. 3) is subtelocentric whereas the other (no. 4) is submetacentric, number 5 is submetacentric, and number 6 is subtelocentric. All six pairs of smaller chromosomes are metacentric or submetacentric, and one of them (*ca.* no. 10 or 11, illustrated as no. 11 here) consistently bears a secondary constriction followed by a satellite near the distal end of the long arm. No heteromorphic pairs of chromosomes were evident in these two males.

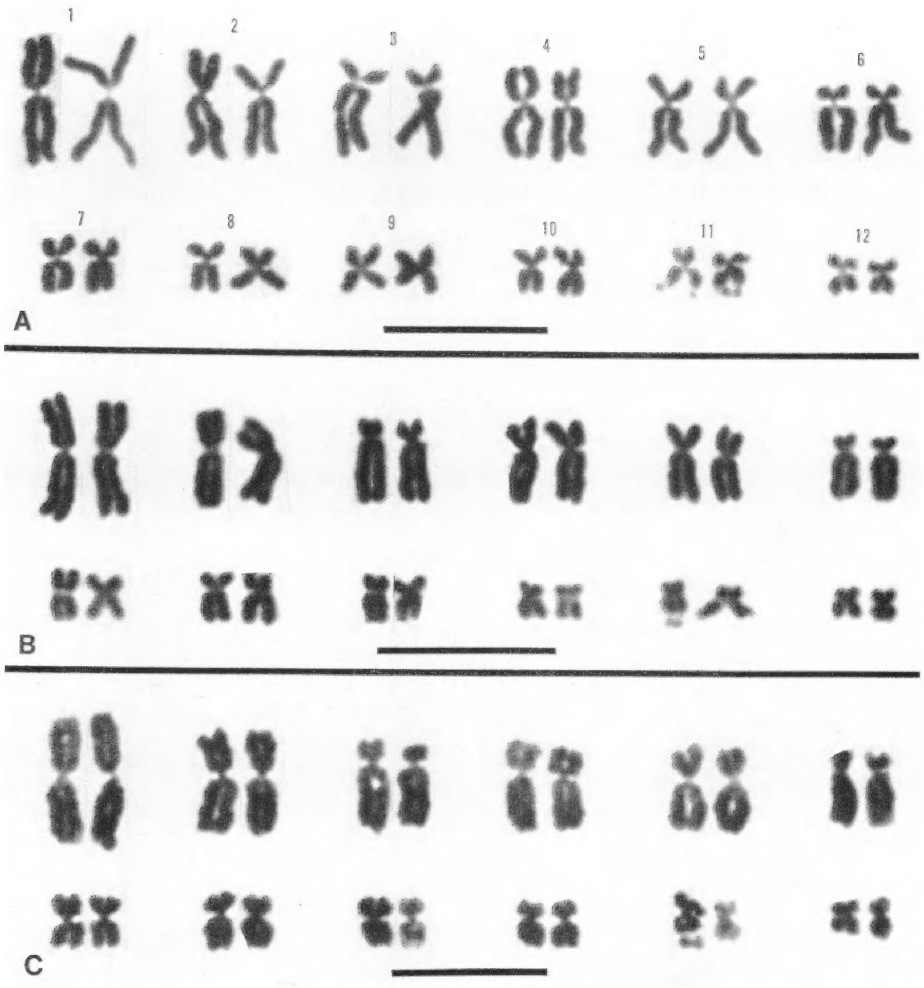

FIG. 1. Karyotypes of three species of hylid frogs. A. *Smilisca baudini* ($2n = 24$), AMNH 84281, male. B. *Pternohyla fodiens* ($2n = 24$), UAZ 30382, male. C. *Triprion petasatus* ($2n = 24$), UAZ 30201, female. Short lines represent 10 μ.

Pternohyla fodiens Boulenger also has a diploid number of 24 chromosomes, of which 12 are rather large and 12 are relatively small (fig. 1B). This was determined by examining complete chromosome complements of 28 cells from four individuals (all males). The karyotype of this species is identical with that of *Smilisca baudini* in all details discernible to me by scrutinizing the chromosomes, including the position of the satellites.

Triprion petasatus (Cope) likewise has a diploid number of 24 chromosomes, of which 12 are rather large and 12 are relatively small (fig. 1C). This was determined by examining complete chromosome complements of 19 cells from four individuals (three males, one female). The karyotype of this species is identical with those of both *Smilisca baudini* and *Pternohyla fodiens* in all details discernible to me. In addition, no heteromorphic pairs of chromosomes were evident in either sex in *T. petasatus*.

The karyotype of *Hyla septentrionalis* Boulenger (fig. 2A) was determined by examining chromosomes of 41 cells from seven individuals (two males, five females). This karyotype ($2n = 24$) also appears to be identical in all respects with those of the three species representing three distinctive genera considered above.

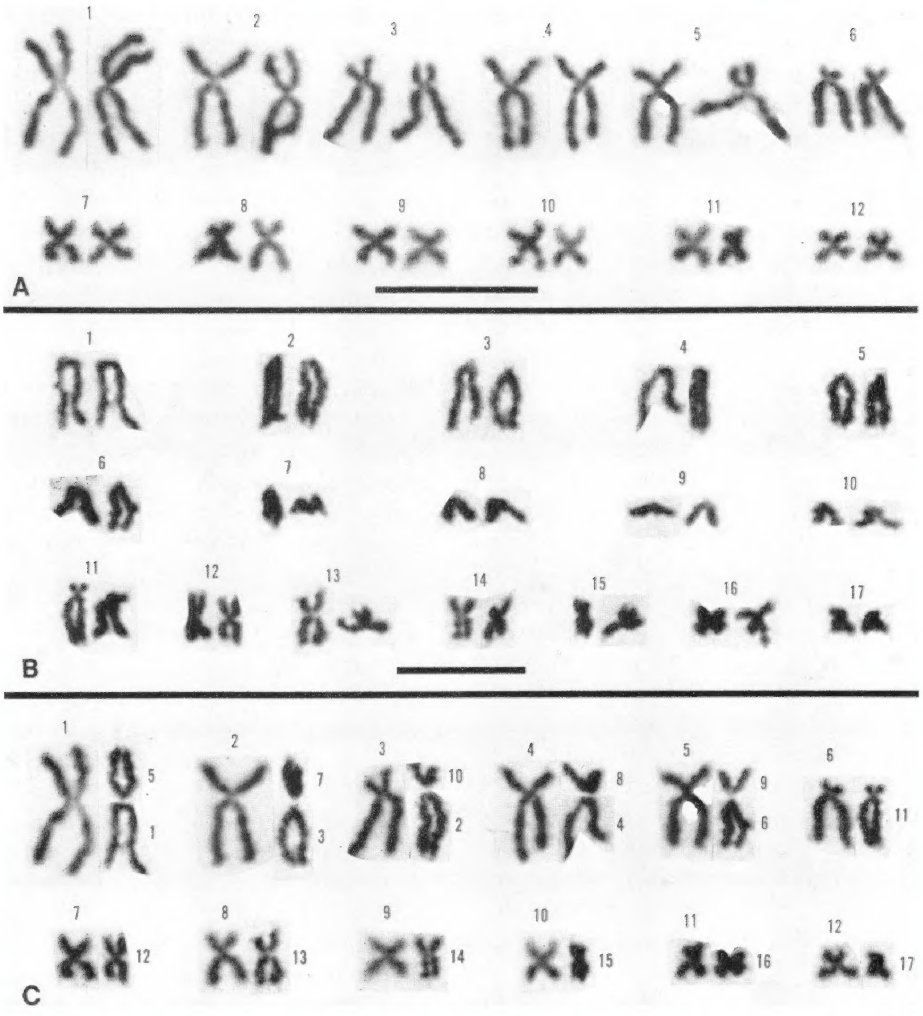

FIG. 2. Chromosomes of hylid frogs. A. Karyotype of *Hyla septentrionalis* ($2n = 24$), AMNH 84166, male; short line represents 10 μ. B. Karyotype of *Hyla brunnea* ($2n = 34$), AMNH 84751, male; short line represents 10 μ. C. A haploid complement from *Hyla septentrionalis* (left chromosome of each "pair"; from the same cell used in A) and *Hyla brunnea* (right chromosome[s] of each "pair"; from the same cell used in B) arranged to illustrate that the karyotype of one species could evolve to resemble that of the other by means of five whole-arm chromosomal rearrangements.

Hyla brunnea Gosse is very distinctive in chromosome number and karyotype (fig. 2B). This was determined by examining chromosome complements of 29 cells from three individuals (one male, two females). This species has a diploid number of 34 chromosomes with a gradual transition in sizes from the largest pair to the smallest such that they are not readily classified into size-groups. The chromosomes are, however, readily classified into two groups on the basis of shapes: 20 are uni-armed (telocentric) and 14 are bi-armed, including one pair (designated here as no. 11) that is subtelocentric and six pairs (nos. 12 through 17) that are metacentric or submetacentric. Neither consistent secondary constrictions nor heteromorphic pairs of chromosomes were evident in either sex, but satellites similar to those occurring in the other species were seen on one of the smallest pairs in one cell.

The karyotypes of *Hyla septentrionalis*

($2n = 24$) and *Hyla brunnea* ($2n = 34$) are distinctly different from one another. Not only is there a difference of 10 in chromosome number, but also the large bi-armed chromosomes that characterize *H. septentrionalis* (and the other three species considered above) are lacking in *H. brunnea*. Instead, *H. brunnea* possesses 20 uni-armed chromosomes (telocentrics), which are entirely lacking in the other species. There are, however, the following important similarities between the karyotypes of *H. brunnea* and the other species: 1) The 12 smallest chromosomes in *brunnea* appear to be the same as those in the other species, perhaps including the pair bearing the secondary constriction; 2) the largest pair of bi-armed chromosomes in *brunnea* (no. 11) appears to be the same as pair number 6 in the other species, which is indicated not only by its shape (subtelocentric), but also by its size; and 3) all the 20 uni-armed chromosomes in *H. brunnea* appear to correspond on a one-to-one basis with the 20 arms comprising the 10 large bi-armed chromosomes in the other species (fig. 2C). These correlations indicate that the karyotype of *H. brunnea* could have been derived from that of *H. septentrionalis* (or a common ancestor; or vice versa) by means of a minimum of five whole-arm chromosomal aberrations. The question is, which karyotype represents the ancestral condition and which the derived? I favor the hypothesis that the karyotype of *H. brunnea* is derived (see discussion below).

DISCUSSION

Hyla septentrionalis and *Hyla brunnea* are very similar in external morphology, and thus there has been some question as to whether they are specifically or subspecifically related. Recently these forms were considered by Duellman and Crombie (1970) and by Trueb (1970), who concluded that they are specifically distinct. The differences in the karyotypes of these forms (fig. 2) are consistent with this conclusion.

Diploid chromosome numbers previously known for species of hylid frogs range from 18 to 30 (excluding polyploid species; e.g., Rabello, 1970; Wasserman, 1970; Bogart, 1973). Thus, the diploid number of *Hyla brunnea* ($2n = 34$) is the highest non-polyploid number now known in the family Hylidae.

Most hylids for which chromosomes have been examined have a diploid number of 24. This includes a variety of frogs with numerous divergent adaptations as well as species occurring in both the Old World and the New World. Karyotypes, in addition to chromosome counts, have been published for several of these species having 24 chromosomes (see list below). Judged from the descriptions and photographs published, the karyotypes of most of these species are remarkably similar to those presented here for the species with 24 chromosomes, even in details such as the number of chromosomes within the different size-groups, positions of the centromeres, and often position of the satellites. In addition, the haploid number of 12 has been reported for two other casque-headed species, *Anotheca coronata* and *Triprion spatulatus* (see Duellman and Cole, 1965), and a diploid number of 24 has been reported for a casque-headed *Trachycephalus* (species not identified; Bogart, 1973).

I mentioned above the similarities and differences between the distinctive karyotype of *H. brunnea* ($2n = 34$) and the more usual karyotype such as that of *H. septentrionalis* ($2n = 24$), indicating that the evolution of one karyotype from the other involved whole-arm chromosomal aberrations (fig. 2C). The question is, which karyotype represents the ancestral condition and which is derived? In most instances when there is a choice involving karyotypic differences such as these, cytologists favor the view that the ancestral karyotype is the one with the higher number of chromosomes (including telocentrics) and that evolution involved "centric fusions," or more specifically, whole-arm translocations between two nonhomologous telocentric chromosomes resulting in the creation of one large bi-armed chromosome. Generally this choice is favored for several reasons: 1) A reasonable mechanism has been recognized for this kind of chromosome mutation; 2) in some specific instances analyzed, aberrations occurring in nature are explained best with this theory; 3) in some groups that were studied in very general terms in the past, including frogs, there appeared to be correlations between relatively high chromosome numbers, relatively more uni-armed chromosomes, and a recognition of being "primitive" based on other characteristics (e.g., skeletal) as determined by

List of Species of Hylid Frogs Having a Diploid Number of 24 Chromosomes and Whose Karyotypes Have Been Illustrated Previously

Species	Reference
Aparasphenodon brunoi (a casque-headed species)	Bogart, 1973
Hyla albofrenata (certain populations)	Bogart, 1973
H. albomarginata	Becak, 1968
H. andersoni	Wasserman, 1970
H. arborea	Seto, 1964; Morescalchi, 1965; Bogart, 1973
H. brieni	Bogart, 1973
H. cadaverina (= *H. californiae*)	Maxson and Jameson, 1968
H. catharinae	Bogart, 1973
H. chrysoscelis	Bogart and Wasserman, 1972; Bogart, 1973
H. crepitans	Rabello, 1970; Bogart, 1973
H. faber	Becak, 1968
H. fuscomarginata	Rabello, 1970
H. granosa	Bogart, 1973
H. hayi	Becak, 1968
H. multilineata	Becak, 1968
H. pardalis	Bogart, 1973
H. perpusilla	Bogart, 1973
H. polytaenia	Rabello, 1970; Bogart, 1973
H. pulchella	Becak, 1968; Bogart, 1973
H. punctata	Bogart, 1973
H. raniceps	Rabello, 1970
H. regilla	Morescalchi, 1965; Maxson and Jameson, 1968
H. rostrata	Bogart, 1973
H. rubra	Bogart, 1973
H. squalirostris	Barrio and Pistol de Rubel, 1970
H. squirella	Bogart, 1973
Phrynohyas venulosa	Rabello, 1970; Bogart, 1973
Pseudacris clarki	Bogart, 1973
Pseudacris streckeri	Bogart, 1973
Smilisca baudini (= *Hyla baudini*)	Bogart, 1973
S. sordida (= *Hyla sordida*)	Bogart, 1973

even earlier workers; and 4) the theory is appealing because it suggests that evolution of karyotypes tends to involve a reduction in chromosome number—or, increased linkage between genes, which would be expected to accompany evolutionary specialization, assuming that natural selection favors the most successful gene combinations (Wickbom, 1950; Matthey; 1951; Patterson and Stone, 1952; White, 1954; Jackson, 1971). On various occasions (e.g., Lowe et al., 1970b) colleagues and I have recognized this as the most likely course of karyotype evolution, particularly when interpreted with consideration of additional biological data for the animals involved.

In some instances, however, there are indications that karyotype evolution occurred in the opposite direction, i.e., two smaller uni-armed chromosomes were derived from a single, larger bi-armed chromosome. This often is referred to casually as "centric fission," although a generally accepted mechanism, particularly for the creation of additional viable centromeres, has not been proposed. Webster, Hall, and Williams (1972) chose centric fission as the most likely explanation for interspecific karyotypic differences they observed in some West Indian lizards of the genus *Anolis*. Also, examples in which a large metacentric chromosome bearing a marker evolved into two smaller telocentric chromosomes were illustrated in lizards of the genus *Cnemidophorus* by Lowe et al. (1970a). The

latter examples are especially instructive because they involve intraspecific variation within a triploid, parthenogenetic all-female species for which the ancestral karyotype is known with virtual certainty.

The case of the hylid frogs considered here appears rather similar to that of the West Indian anoles considered by Webster, Hall, and Williams (1972). Namely, nonkaryotypic information about the animals suggests that the species (*H. brunnea*) having the distinctive karyotype with an unusually high chromosome number and numerous telocentrics is a relatively recently derived form (Trueb, 1970). Thus, one may infer that the karotype of *Hyla brunnea* ($2n = 34$) was derived from a karyotype such as that of *Hyla septentrionalis* ($2n = 24$) by means of "centric fission." This conclusion is supported by the following indications: 1) Many hylids from several continents and with widely diverse adaptations have a diploid number of 24 chromosomes that are morphologically similar to those of *Hyla septentrionalis*; 2) this karyotypic pattern occurs in several casque-headed hylids as well as in species lacking such cephalic specializations; 3) a haploid chromosome number of 12 has been determined for all six species in the genus *Smilisca* (Duellman and Trueb, 1966); 4) karyotypes similar to those known to occur in the various genera and species considered here also may occur in the additional closely related species for which only chromosome numbers ($n = 12$, $2n = 24$) are known, and thus it would be most consistent with the phylogenetic tree proposed by Trueb (1970, p. 699) if such a karyotype occurred in the most primitive species indicated in her figure; 5) *Hyla crepitans*, *Hyla faber*, and *Hyla pardalis*, members of the South American *Hyla boans* group that Trueb (1970) considers as possibly ancestral to the West Indian *septentrionalis* group (which includes *H. brunnea*), also have generally similar karyotypes with 24 chromosomes (Becak, 1968; Rabello, 1970; Bogart, 1973); and 6) the only other species (*Hyla rosenbergi*) of the *boans* group whose chromosomes have been counted has a haploid number of 12 (León, 1969 [1970]).

The *septentrionalis* species group as considered by Trueb (1970) includes five species, of which chromosomes have been examined for only *H. brunnea* and *H. septentrionalis*. Although there are no chromosome data for the remaining three species (*H. dominicensis, H. lichenata*,[1] and *H. vasta*), a few predictions can be made concerning their karyotypes. This is reasonable because karyotype variation generally is rather conservative in frogs and a phylogeny of the group based on other characteristics has been proposed recently (Trueb, 1970, p. 699). Thus, if the interpretation of chromosome evolution presented here and the pertinent parts of Trueb's phylogeny are correct, *Hyla vasta* and *Hyla dominicensis* may prove to have karyotypes very similar to, or indistinguishable from, that of *Hyla septentrionalis*, whereas the karyotype of *Hyla lichenata* may be rather different. That is, of the species in the *septentrionalis* group whose chromosomes have not been examined yet, *Hyla lichenata* may be the most distinctive in karyotype.

The alternative hypothesis that the karyotype of *H. brunnea* ($2n = 34$, with many uni-armed chromosomes) represents the ancestral condition should not be totally ignored. The chromosomes of many additional species are yet to be examined, with the realization that karyotypes may be extremely conservative and that often relics of primitive forms occur in isolated populations on islands.

SPECIMENS EXAMINED

All specimens (N = 20) from which chromosomes were examined are catalogued individually in the herpetological collection at either the American Museum of Natural History (AMNH) or the University of Arizona (UAZ); catalogue numbers are in parentheses.

Smilisca baudini (N = 2; both males): MEXICO: *San Luis Potosí*: 35.6 mi. (by Highway 85) S Ciudad de Valles (AMNH 84280). *Veracruz*: 21.9 mi. (by Highway 140) SE Jalapa (AMNH 84281).

Pternohyla fodiens (N = 4; all males): MEXICO: *Sonora*: 1 km. (by road) S center of Navojoa (UAZ 30380-30382 and 30385).

Triprion petasatus (N = 4; three males, one

[1]Schwartz and Fowler (1973) used the name *Hyla crucialis* Harlan for this species, which is explained by Crombie (1973). The hylid frogs of the Greater Antilles were reviewed most recently by Trueb and Tyler (1974).

female): MEXICO: *Yucatan*: E edge Merida on Highway 180 (UAZ 30167, 30168, 30201, and 30202).

Hyla septentrionalis (N = 7; two males, five females): UNITED STATES: *Florida*: Collier Co.: Naples (AMNH 84165, 88003, 88004, 88006, and 88007). Monroe Co.: Key West (AMNH 84166 and 84322).

Hyla brunnea (N = 3; one male, two females): JAMAICA: *Trelawny Parish:* Barbecue Bottom, *ca.* 9 mi. S Duncans (AMNH 84749, 84751, and 84752).

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